UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

003269

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Caswell #453B
Shaughnessy #113101

MEMORANDUM

TO: Richard Mountfort, PM#23
Registration Division (TS-767C)

SUBJECT: Sonalan (ethalfluralin technical, Lilly compound 94961): Evaluation of Genetic Toxicology Studies.
EPA Reg.#1471-122 EPA Accession No.: 250475

Two bacterial and three mammalian mutagenicity studies, submitted June 9, 1983 by ELANCO PRODUCTS (Division of Eli Lilly & Co.), were reviewed and evaluated, as follows:

(1) Bacterial Mutation Study 830401GPA1169 (ACCEPTABLE)

(2) Bacterial Mutation Studies: 830307AMS1169; 830404AMS1169; and 830430AMS1169 (ACCEPTABLE)

(3) Hepatocyte DNA Hepatocyte Repair Study 791Y20-263 (ACCEPTABLE)

(4) L5178Y TK Foreward Mutation Study 830214SCE1169 (ACCEPTABLE)

(5) Sister Chromatid Exchange Study 830214SCE1169 (NOT ACCEPTABLE)

Studies (1), (2), (3) and (4) are judged ACCEPTABLE; study (5) is NOT ACCEPTABLE, since only females were sampled.

Individual Data Reviews for these studies are attached.

Irving Mauer, Geneticist
Toxicology Branch/HED (TS-769C)

09-26-83

DC
9/27/83

9/28/83

TOXICOLOGY BRANCH: DATA REVIEW

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Caswell No.: 453B
Shaughnessy No.: 113101

Chemical: SONALAN (ethalfluralin)

Study Type: Gene mutation in bacteria (gradient plate assay)

Citation: The Effect of Ethalfluralin on the Induction of Bacterial Mutation Using a Modification of the Ames Test

Accession No./MRID No.: 250475/(NA)

Sponsor/Contracting Lab.: Toxicology Division, Lilly Research Laboratories Division of Eli Lilly and Company, Greenfield, Indiana 46140

Report No./Date: Study 830404GPA1169, June 1983

Test Material: Ethalfluralin technical, Lilly compound 94961, Lot #B30-Y64-35B, 95.5% a.i.

Procedures: Eight his- strains of Salmonella typhimurium and two try- strains of Escherichia coli were simultaneously treated with test substance (in DMSO) in a modification of the Ames procedure involving a 10,000-fold range of gradient concentrations (0.1 to 1000 ug/ml), both in the absence and presence of metabolic activation provided by microsomal fractions of liver (S-9) from rats primed with Arochlor 1254 (induced S-9), as well as those from non-induced (normal) animals.

Results: Increases in the incidence of revertent colonies (positive results) were obtained in non-activated cultures of TA1535, TA100 and TA98, in activated (induced S-9) cultures of TA1535 and TA100, and in non-induced S-9 cultures of TA100 --- but only in the highest of gradient plates, 100-1000 ug/ml.

Conclusions: Since all cultures responded adequately to the positive control substances (MNNG and 2AA), and appropriate procedures were employed throughout, the conclusion that ethalfluralin technical is weakly mutagenic in bacterial cultures of S. typhimurium and E. coli is supportable.

Core Classification: Acceptable.

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09/26-83

TOXICOLOGY BRANCH: DATA REVIEW

003269

Caswell No.: 453B
Shaughnessy No.: 113101

Chemical: SONALAN (ethalfluralin)

Study Type: Gene mutation in bacteria (Ames Assay)

Citation: The Effect of Ethalfluralin (Compound 94961) on the Induction of Reverse Mutations in Salmonella Typhimurium Using The Ames Test

Accession No./MRID No.: 250475/(NA)

Sponsor/Contracting Lab.: Toxicology Division, Lilly Research Laboratories Division of Eli Lilly and Company, Greenfield, Indiana 46140

Report No./Date: Studies 830307AMS1169, 830404AMS1169, and 830425AMS1169; May 1983

Test Material: Ethalfluralin technical, Lilly compound 94961, Lot #B30-Y64-35B, 95.5% a.i.

Procedures: Five his- strains of Salmonella typhimurium (TA1537, TA1538, TA1535, TA98, TA100) were treated with test compound (in DMSO) by the standard Ames plate incorporation procedure at dose levels ranging from 125 to 1000 ug/plate (based upon toxicity and precipitation tests at the HDT), both in the absence and presence of metabolic activation provided by microsomal fractions prepared from livers (S-9) of non-induced (normal) rats, as well as those pre-treated with Arochlor 1254 (induced S-9). Parallel cultures were exposed to the mutagen (positive controls) appropriate for each strain (MNNG, 2NF, 9AmAc for non-activated testes) and mode (2AA for all activated tests).

Results: Only in replicate activation tests with induced S-9 in TA1535 and TA100 cultures were positive results found (>2-fold revertants compared to DMSO control values) ; a dose-response was obtained only for TA100 tests. Only TA100 responded (in a non dose-related manner) in non-induced S-9 cultures.

Conclusions: Since all cultures responded to the positive control substances (mutagens) in a satisfactory manner, and appropriate procedures were employed throughout, the conclusion that ethalfluralin induced point mutations (base-pair mutations) in strains TA100 and TA1535 in the presence of a metabolic activation enzyme system, but is of a low order of potency (= "weakly mutagenic"), is supportable.

Core Classification: ACCEPTABLE

09.26.83

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(3a)

SONALAN® E.C. [Ethalfluralin]
EPA File Symbol No. 1471-REE
EPA Acc.#250475, submitted June 9, 1983

Caswell No.: 453A
Shaughnessy No.: 113101

NB:

The report "The Effect of Ethalfluralin (Lilly Compound 94961) on the Induction of DNA Repair Synthesis in Primary Cultures of Adult Rat Hepatocytes (Study 791120-263)" was apparently submitted to the U.S. EPA at an earlier date. It is included in the registrant's present submission (EPA Acc.#250475), because of "its relevance to the current genetic toxicology studies." This study is reviewed with this submission, because it apparently was not reviewed at the time of its initial submission.

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(3b)

TOXICOLOGY BRANCH: DATA REVIEW

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Caswell No.: 453B
Shaughnessy No.: 113101

Chemical: SONALAN (ethalfluralin)

Study Type: (in vitro) Rat hepatocyte DNA repair (HPC-UDS)

Citation: The Effect of Ethalfluralin (Lilly Compound 94961) on The Induction of DNA Repair Synthesis in Primary Cultures of Adult Rat Hepatocytes

Accession No./MRID No.: 250475/(NA)

Sponsor/Contracting Lab.: Toxicology Division, Lilly Research Laboratories Division of Eli Lilly and Company, Greenfield, Indiana 46140

Report No./Date: Study 791120-263, June 1980

Test Material: Ethalfluralin technical, Lilly compound 94961, Lot #B30-Y64-35B, 93.5% a.i. (GC).

Procedures: Primary hepatocyte cultures from an adult (165g) male F344 rat (HPC) were incubated for 20 hr with graded concentrations of test material dissolved in DMSO (0.5, 1.0, 5, 10, 50, 100, 500, 1000 nM/ml) plus 10 uCi/ml tritiated thymidine according to published methods. Coverslip preparations were stained (1% aceto-orcein), air-dried and treated for the determination of unscheduled DNA synthesis (UDS) under oil-immersion microscopy. HPC-UDS was quantified by automated counting of net nuclear silver grains (minus cytoplasmic background) over 20 morphologically unaltered cells per test dose up to the highest non-toxic concentration.

Results: Ethalfluralin technical was inactive in inducing UDS at all levels of ethalfluralin technical up to cytotoxic doses (500 and 1000 nM).

Conclusions: The negative result for the test material in this assay is substantiated by adequate protocol and control procedures, including positive results (induction of UDS) by the mutagens MNNG (ultimate carcinogen) and 2-AAF (pro-carcinogen).

Core Classification: ACCEPTABLE.

Irving Mauer, Geneticist
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09.26.83

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TOXICOLOGY BRANCH: DATA REVIEW

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Caswell No.: 453B
Shaughnessy No.: 113101

Chemical: SONALAN (ethalfluralin)

Study Type: (in vitro) Gene mutation in mammalian cells (Mouse Lymphoma Assay).

Citation: The Effect of Ethalfluralin (Compound 94961) on the Induction of Forward Mutation at The Thymidine Kinase Locus of L5178Y Mouse Lymphoma Cells

Accession No./MRID No.: 250475/(NA)

Sponsor/Contracting Lab.: Toxicology Division, Lilly Research Laboratories Division of Eli Lilly and Company, Greenfield, Indiana 46140

Report No./Date: Study 830208MLA1169, April 1983

Test Material: Ethalfluralin technical, Lilly compound 94961, Lot #B30-Y64-35B, 95.5% a.i.

Procedures: Cleansed cultures (free of spontaneous mutants) of the subline TK3.7.2C derived from the mouse lymphoma cell line L5178Y, heterozygous for the thymidine kinase locus ($TK^{+/-}$), were exposed to test material in DMSO for 4 hr at doses of 0.1, 0.25, 0.5, 0.75, 1.0, 2.5, 5.0 and 10 ug/ml (based upon preliminary cytotoxicity testing which indicated greater concentrations decreased cell growth to <10%), both in the absence and presence of a metabolic activation system derived from a hepatic microsomal enzyme system prepared from male F344 rats treated with Aroclor 1254 (induced S-9), according to established (published) methods. The cultures were then incubated for 48 hr (expression time), and cloned for $TK^{+/-}$ mutants in the presence of trifluorothymidine (TFT) which preferentially selects for only mutant colonies. The induction of viable mutants was automatically quantitated (Biotran II Colony Counter) in triplicate sample determinations for each treatment, and cultures showing >10% survival evaluated for mutagenic response.

Results: At dose-dependent cytotoxic levels of 34-81% in non-activated cultures and 25-158% in activated cultures, ethalfluralin was judged not to induce increased incidences of mutants ($TK^{-/-}$) at any concentration, in contrast to positive results (induction of increased mutation) obtained with 620 ug/ml EMS (direct mutagen) or 5 ug/ml 3-MC (pro-carcinogen).

Conclusions: The negative result for mutagenicity obtained for ethalfluralin under the conditions of this test (i.e., no evidence of induced mutation in L5178Y cells) is supportable by adequate protocol and control procedures.

Core Classification: ACCEPTABLE.

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09.26.83

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TOXICOLOGY BRANCH: DATA REVIEW

003269

Caswell No.: 453B
Shaughnessy No.: 113101

Chemical: SONALAN (ethalfluralin)

Study Type: (in vivo) DNA-repair in Chinese hamsters (BM-SCE)

Citation: The Effect of Ethalfluralin (Compound 94961) on The in vivo Induction of Sister Chromatid Exchange in Bone Marrow of Chinese Hamsters

Accession No./MRID No.: 250475/(NA)

Sponsor/Contracting Lab.: Toxicology Division, Lilly Research Laboratories Division of Eli Lilly and Company, Greenfield, Indiana 46140

Report No./Date: Study 830214SCE1169, March 1983

Test Material: Ethalfluralin technical, Lilly compound 94961, Lot #B30-Y64-35B, 95.5% a.i.

Procedures: Inbred adult (30-40g) female Chinese hamsters (Lilly Lab. Colony) were implanted subcutaneously with bromodeoxyuridine (BUdR) tablets, and 5 hr later groups of 3 animals each given acute 10 ml/kg oral doses of test substance (200, 300, 400, and 500 mg ethalfluralin/kg body wt). Nineteen hr later, femoral bone marrow was removed from Velban-treated animals (mitotic-arresting agent), and prepared for metaphase analysis for sister-chromatid exchanges (SCE), according to established methods (slides stained with Hoechst 33258, exposed to uv, and processed with SSC and Giemsa stain). Twenty-five metaphases from each animal demonstrating the typical second-division SCE differentiation were scored and compared to controls.

Results: Even at cytotoxic doses of 400 and 500 mg/kg, ethalfluralin technical did not induce SCE above controls, in contrast to the positive control pro-mutagen, 50 mg/kg cyclopnosphamide which gave SCE values significantly different from controls (DMSO/10% aqueous acacia).

Conclusions: The negative result for in vivo induction of DNA damage/repair (SCE in BM of female Chinese hamsters) is substantiated by adequate protocol and control procedures for one sex only.

Core Classification: NOT ACCEPTABLE. Tests with males are also required, and it is recommended that spermatogonial metaphases also be sampled for SCE.

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09.30.83

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